BACTERIOPHAGE AND HOST BACTERIA INTERACTIONS WITHIN THE ALA WAI CANAL

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ABSTRACT

Bacteriophages, or also known as phages, are viruses that infect specific types of bacteria and have major influence on the ecology of bacteria. Phages can lyse and kill bacteria, mediate horizontal gene transfer between bacteria, or establish symbiotic relationships with bacteria that change the behavior of the host. As a first step towards understanding how phages influence the ecology of bacteria within the Ala Wai canal, a drainage canal in Waikiki often used for recreation, we isolated and characterized phage-host systems. Of 25 distinct bacteria in three different phyla isolated from the canal (22 Proteobacteria, 2 Firmicutes, and 2 Bacteroidetes), we detected phages that infected five of them (all Gammaproteobacteria) using the agar overlay technique. The host range test and restriction enzyme digests indicated that all five phages were distinct and specific to the host on which they were isolated. Electron microscopy of lysed bacteria cultures revealed phage-like particles with capsid diameters in the range of 50-75 nm, at least one of which had a tail suggesting it belongs to the viral family *Caudovirales.* This study demonstrates that phages are readily detectable in the canal but additional work would be needed to determine both their abundance and influence on bacterial populations in the canal.

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1.0. INTRODUCTION

1.1. Background

Bacteriophages, commonly known as phages, are viruses that infect, kill, and lyse the bacteria that they infect (Duckworth & Gulig, 2002). Phages first infect bacteria by binding to a specific receptor on the host cell surface (Duckworth & Gulig, 2002). The host range of a given phage is often limited to a single species of bacterium because of the highly specific nature of the binding (Duckworth & Gulig, 2002). Once attached, the phage injects its genome into the host bacterium where the genes are expressed. Within the host cell, the phage genome is replicated and the structural components are produced, ultimately leading to death of the host cell by lysis. This will then release hundreds of new phages back into the environment (Duckworth & Gulig, 2002). The cycle can then be continued by the newly produced phages infecting other host bacteria cells. The lysis of bacteria by phage on an agar plate shows up in the form of a clear spot known as a plaque (Duckworth & Gulig, 2002).

Phages can be characterized by various criteria including infection phenotype, virion morphology, and genome sequence (Abedon, 2008). In terms of infection types, there are generally two types of phages: lytic and temperate. Lytic phages are only replicating via the lytic cycle in which the phage directs the production of new phage particles and destroys the host bacterium (Abedon, 2008). Temperate phages, on the other hand, are capable of replicating by either the lytic cycle or the lysogenic cycle (Abedon, 2008). Phage morphology can vary between tailed or binary, isometric, helical, and pleomorphic (Abedon, 2008).

Viruses in aquatic ecosystems range in concentration from 10^3 to over 10^8 per milliliter of water (Boehme et al., 1993) and are a major source of mortality for bacteria (Wommack et al 2000). Phages play a role in the aquatic microbial loop as well as affect bacteria diversity (Abedon, 2008). In aquatic ecosystems, bacteria make a large contribution to primary production, which makes organic carbon available to higher trophic levels in the ecosystem (Abedon, 2008). However, phage lysis can result in the recycling of photosynthetically fixed carbon from particulate to dissolved organic material (Abedon, 2008). Phage infections of bacteria can also result in modification to the bacterial genotypes through transduction. In transduction, genetic material from one bacterium can be packaged in a phage particle and be transferred to another (Abedon, 2008). Thus, phage-host interactions can increase genetic diversity in bacteria.

The Ala Wai canal is a drainage canal for the Manoa, Palolo, and Makiki watersheds. These three streams as well as surface runoff and outflow from storm drains flow into the canal, through the Ala Wai harbor, and to the ocean. Although the canal is not designated as a recreational waterway by the state, it is frequently used for paddling and fishing; therefore the microbial water quality is of concern. A number of studies have looked at pathogenic bacteria in the canal, but little is known about the role of bacteriophages. As a first step towards understanding the ecology of bacteria we isolated and characterized phage-host systems in the Ala Wai canal.

1.2. Plaque Assays

Studies of bacteriophages often use a technique known as plaque assays as a way to measure the abundance of phage in a sample (Juarez et al., 2013). For this research, plaque assays were the method of choice to find phages from environmental water samples. The plaques that appear on a plaque assay plate are clearings where cells have died due to the viral infection of the host cell (Baer & Kehn-Hall, 2014). The easily identifiable spots on the plate make phage isolation and phage quantification relatively simple. During a plaque assay, a layer of bacteria host cells is infected with an unknown concentration of lytic phage that has been serially diluted (Baer & Kehn-Hall, 2014). The serial dilution of the phage results in a countable range of plaques.

After the initial infection, plaque spots will begin to form as the replication-lysis-infection cycle continues (Baer & Kehn-Hall, 2014). Over time, the plaques will become distinct and will show their morphology. Plaque morphology can vary between growth conditions and viral species (Baer & Kehn-Hall, 2014). Plaque size, clarity, border definition, and distribution can provide information on the virus in question, as they may also distinguish different phages on a single agar plate (Baer & Kehn-Hall, 2014).

1.3. Objectives of Study

Within the Ala Wai canal here on Oahu, the genetic relationships as well as the diversity of phage-host systems are unknown. To begin to understand phage-host interactions within the Ala Wai canal the following objectives were set:

1) Isolate marine bacteria from Ala Wai water samples
2) Conduct plaque assays to find phages that infect the isolated bacteria
3) Determine similarities/differences between isolated phages by restriction enzyme digest and gel electrophoresis
4) Conduct plaque assays to determine the host range of infection the isolated phages have with the isolated bacteria
5) Use PCR to genetically identify the bacteria
6) Obtain images of phages using electron microscopy

2.0. METHODS

2.1. Sample Collection

All water samples were collected from the same site in the Ala Wai Canal, east of the Ala Wai Boat Harbor (21.287685 N, 157.839783 W) in 1 liter (L) HDPE bottles. The samples from which the bacteria were isolated from were collected in three, 1 L HDPE bottles on October 9, 2017 between 10:19am and 10:31am. The two, 1 L samples from which phages were found were collected on January 31, 2018 at 10:15am.

2.2. Medium

An estuarine heterotrophic bacterial medium (EHBM) was prepared from a base 1:1 mixture of freshwater (collected from Manoa-Palolo Stream) and seawater (collected from Ala Wai Harbor), which are end members for the waters of the Ala Wai Canal. The waters were filtered (0.2 μm polyethersulfone membrane), stored at 4 °C, and filtered again prior to use. The base mixture of freshwater and seawater was combined with Bacto™ agar, Bacto™ peptone, and Bacto™ yeast powders for ideal marine bacteria growth conditions. The final mixture resulted with a salinity of about 18 parts per thousand (ppt). For plates, agar was added at 1.5% final concentration. The media was mixed and autoclaved on a liquid cycle for 40 minutes for sterilization. Pouring of the plates was done within a sterile fume hood into sterile VWR petri dishes or plates, with each receiving 15 mL of agar. To make marine top agar and marine broth, the same steps were used for the process of agar plates except for the amount of agar required. For top agar, the amount of agar used was 0.6% of the total volume in contrast to the 1.5% for agar plates. For the marine broth, agar was simply not added.

2.3. Sample Plating

After sample collection, bottles were taken back to the Model Systems lab in CMORE Hale for seawater plating. To begin the bacteria isolation process, 100 microliters (μL) of fresh Ala Wai water samples were used for plating onto marine agar plates. The plates were then left in an incubator overnight set at 37 °C. Plates were checked the following day for colonies. Using 100 μL of sweater resulted in slightly crowded plates with bacteria colonies occasionally growing into each other. Because of this, following sample collections were plated using 75 μL and 50 μL of water for colony picking.

2.4. Bacteria Colony Picking

Cells from a total of 25 colonies, chosen to represent diversity in characteristics such as size, shape, color, and texture, were picked and transferred to new plates using a sterile inoculating loop. After the initial picking, each colony was streak plated onto marine agar plates as part of the bacteria isolation process. After streak plating the plates were set in the incubator overnight for growth. The following day single colonies within the streak plates were picked and re-streaked onto new plates for further isolation. This process was repeated two additional times to ensure that each plate held only one type of bacteria.

2.5. Bacteria Stocks

After streak purification, triplicate frozen glycerol stocks were made for each isolate by mixing 250 μl of sterile glycerol per 1 mL of dense culture (grown overnight at

26 °C, 250 rpm) and storing at -80 °C. To confirm viability of the cells in the stocks, material was scraped directly from the frozen stock with a sterile loop, streaked onto EHBM agar, and the plates examined for growth after overnight incubation at 37 °C.

2.6. Phage Detection

The method used to detect bacteriophages was via plaque assays using the agar overlay technique. The agar overlay technique combines the bacteria culture and seawater sample (potential phage source) with top agar where it is plated and incubated overnight for growth. This mixture with top agar and bacteria liquid culture results in a "lawn" of bacteria growth on the agar plates. If phages are present, they will show up as clearings within the bacteria lawn on the agar plate.

The process begins with two fresh Ala Wai seawater samples both collected in 1 L bottles the morning phage detection plaque assays were done. The seawater was then filtered with a sterile 0.2-micron filter (Sterivex GP, Millipore) using a peristaltic pump at 120 mL min^{-1}. The seawater was filtered into different 1 L bottles where one was left as filtered seawater (sample 1) while the other was then inoculated with 200 µL of liquid culture of each isolated bacteria in addition to 10 mL of marine broth for nutrients to promote growth (sample 2). After inoculation sample 2 was placed in a shaker rotating at 250 rpm at 26 °C for overnight growth. The following day, sample 2 was filtered again using the same pump set up with a sterile 0.2 µm filter.

2.7. Sample Concentration

After the inoculation process, samples 1 and 2 were concentrated using a 15 mL 100,000 molecular weight centrifugal filter. Because the filter tubes could only hold 15 mL at a time, two filters were used for each seawater sample. 15 mL of each seawater sample was poured into the top reservoir of the filter and spun in a centrifuge at 4000 xG for 10 minutes, two rounds each. Each round resulted in about 150 µL left of sample in the top reservoir. After the two rounds, 250 µL of the filtrate was pipetted into both top reservoirs for sample 1 for recovery. This would then be used for phage spot testing. Sample 2 was recovered after pipetting 5 mL of filtrate into the top reservoir for plaque assay use. Left over filtrate was used as a control during spot tests.

2.8. Plaque Assays / Spot tests

Plaque assay preparation begins with inoculating 3 mL marine broth liquid cultures in sterile 15 mL tubes for all isolates. Each grew overnight within the shaker rotating at 250 rpm at 26 °C. The following day, the liquid cultures were centrifuged at 5300 xG for 10 minutes to bring all the cells to the bottom. 2 mL of supernatant was pipetted out followed by cell re-suspension to increase cell density. Sterile tubes were prepared to hold 3mL of marine top agar in a warm water bath set at 42 °C to keep the top agar in a liquid state. For plaque assays, tubes were prepared with 100 µL of liquid culture combined with 100 µL of water sample 2. Liquid top agar was poured into the sample tubes, quickly vortexed to mix, poured onto marine agar plates, and spread evenly. For plates that would undergo spot tests, 100 µL of liquid culture was poured with top agar without a water sample. After the poured top agar solidifies, 10 µL of

sample 1, sample 2, and control sample were pipetted onto different marked areas of the plates. Once all plating was complete, plates were left overnight in an incubator set at 37 °C for growth.

2.9. Bacteriophage Isolation

Bacteriophage isolation begins with picking isolated phage colonies or plaques present on plates after the initial plaque assay. The bacteria with plaques were numbers 1, 7, 11, and 18. Three days after the initial plaque assay, what appeared to be plaques for bacteria 15 and 21 showed up and were picked for testing. Each bacteria plate with plaques had four-isolated plaque colonies picked and placed into 500µL of SM buffer and vortexed, except for bacteria 15 and 21. Bacteria 15 and 21 had only two samples picked as the potential plaque spots looked different from the others.

2.10. Serial Dilutions

To increase the chances of a plaque colony only containing one specific type of phage, plaque assays are performed using different dilutions of the initially picked phage colonies (stock sample). The serial dilutions used included 10^{-2}, 10^{-4}, and 10^{-6} dilutions. Diluting the stock sample of the picked phage colony results in scattered plaques from plaque assays that are ideal for phage isolation. These newly isolated plaques are then picked and once again placed in 500 µL of SM buffer where they will be diluted and used for another set of plaque assays.

2.11. Phage Isolation Plaque Assays

Phage isolation plaque assays are very similar to the plaque assays already described. The main difference is that the bacteria used were only those with which plaques were picked from in addition to varying phage sources that were mixed with the 100 µL of liquid culture. The phage sources used were for the respective bacteria with which they were picked from. This picking, dilution, and plaque assay process was repeated to achieve a total of three total phage isolation plaque assays.

2.12. DNA Purification

DNA purification begins by generating concentrated phage samples. 500 µL of dense bacteria culture (grown overnight at 26 °C, 250 rpm) was added to 15 mL of marine broth to get a 30x dilution and incubated in a shaker rotating at 250 rpm at 26 °C. After the liquid cultures incubated for two hours, 200 µL of isolated phage samples were added to promote phage replication and incubated once again in the shaker at the same settings. The following day the liquid cultures were centrifuged for 10 minutes at 5300 xG. The supernatant was filtered (Puradisc 0.2 µm filter) followed by concentration via 100,000 molecular weight centrifugal filters spun at 4000 xG for 10 minutes.

150 µL of each concentrated phage sample received 1 unit of DNAse and sat at room temperature for 15 minutes. After 150 µL of 2xT buffer mixed with 1 µL of Proteinase K (50 µg μL^{-1}) was added for each sample and incubated at 65 °C for 30 minutes. Following incubation the samples were quickly cooled at room temperature for 5 minutes. 1 µL of RNAse A (5 µg μL^{-1}) was then added and left at room temperature for 15 minutes. 150 µL of MPC Protein Precipitation Reagent was then added and vortexed

briefly. After samples were sufficiently mixed, they were centrifuged at 13,000 rpm at 4 °C. The supernatant was removed and transferred to a new, clean tube. 400 µL of isopropanol was added and centrifuged again at 13,000 rpm at 4 °C. The supernatant was removed and transferred to another new tube where samples were rinsed twice with 500 µL of 70% ethanol. In between rinses, the samples were centrifuged at the same conditions to remove the supernatant before the final rinse. As much ethanol as possible was carefully removed before placing samples with open caps, covered over with foil, in an incubator at 65 °C to evaporate any residual ethanol. DNA was then re-suspended in 50 µL of Teknova Suspension Buffer.

2.13. DNA Clean Up & Concentration

To ensure purity of the DNA, samples were cleaned and concentrated in membrane columns (Zymo-Spin Columns™). For genomic DNA, a 2:1 ratio of binding buffer to sample was added and briefly mixed by vortexing. The mix of each sample was then transferred to a spin column set in a collection tube. The samples were centrifuged for 30 seconds and the flow-through discarded followed by washes. Two washes were done for each sample, both adding 200 µL of DNA wash buffer to the column and centrifuging the sample for 30 seconds. After the washes, 50 µL of DNA elution buffer was added directly to the column and incubated at room temperature for one minute. To elute the DNA, the column was transferred to a new 1.5 mL microcentrifuge tube and spun for 30 seconds.

2.14. DNA Concentrations

Concentrations of DNA were determined using fluorometry. DNA was prepared using the Invitrogen Quant-iT dsDNA Assay Kit. A standard DNA concentration curve was used (from Quant-iT dsDNA Assay Kit) along with phage DNA samples. For the standard curve, 10 µL of DNA were added to individual wells. For phage samples, 1 µL from phage DNA was added to the wells. After loading the DNA, 1 µL of DNA stain was added for each sample mixed with 200 µL of HS buffer. The wells were then mixed briefly by pipetting before loading into the spectrometer.

2.15. Restriction Enzyme Digest

Enzymes HindIII-HF and EcoRI-HF were used for the enzyme digest of purified phage DNA. The protocol followed (Time-Saver™ Protocol) was relative to a 50 µL total reaction. 1 µL of restriction enzyme is added with 1 µg of sample DNA, 5 µL of 10x NEBuffer, and 47 µL of nuclease-free water for a total of 50 µL. Once prepared, the samples were placed in a water bath set to 37 °C to incubate for 15 minutes. After incubation, 10 µL of 6x gel loading dye was added to stop the reaction. 250 nanograms (ng) of DNA were used for all reactions. Controls were made by using 10 ng of DNA mixed with the NEBuffer and no enzymes.

2.16. Gel Electrophoresis

Agarose gels (0.8% Bioline Molecular Grade) were made using 1x TAE buffer. 20 µL of samples from the restriction enzyme digest were loaded into each well and ran at 80 volts for roughly 6 hours.

2.17. Electron Microscopy

Samples were applied to 200 mesh carbon-coated Formvar-coated copper grids and negatively stained with approximately 2.5% uranyl acetate.

Applying samples to the grids begins with pipetting 4 µL of phage stock samples onto the copper grids and left to sit for 45 seconds. After the 45 seconds, an absorbent paper was used to wick away remaining liquid but careful to make sure that the grid does not dry out. Next, 10 µL of nuclease-free water was pipetted onto the grid as a first wash then wicked away, again being sure not to leave the grid dry. Following the wash, 4 µL of the uranyl acetate stain was added and left to sit for another 45 seconds. Finally after wicking away the stain, 10 µL of nuclease-free water was added as the second and final wash. After the wash, the liquid is wicked down to as thin of a layer as possible without drying the grid completely.

Stained grids were viewed on a Hitachi HT7700 TEM at 100 kV, and photographed with an AMT XR-41B 2k x 2k CCD camera.

2.18. 16S rRNA Gene Sequencing of Bacteria Isolates

Polymerase chain reaction (PCR) was used to amplify the 16S rRNA gene to use for sequencing bacteria. Bacteria DNA samples were first prepared to each have a

concentration of 2 ng/µL so that 5 µL (10 ng) could be used for the PCR mix. The 5 µL of DNA samples were mixed with 5 µL of nuclease-free water, 12.5 µL of 2X Platinum™ SuperFi™ PCR Master Mix, 1.25 µL of 10 µM 16S forward primer, and finally 1.25 µL of 10 µM 16S reverse primer for a PCR reaction total of 25 µL. Two control samples were prepared to check if amplification occurs without the addition of DNA. Amplification of the controls would indicate that our sample preparation was contaminated. An additional bacterium known to be a type of vibrio was included for sequencing bringing the sample total to 26.

PCR samples went through 30 cycles three stages, including a denaturing stage lasting 20 seconds at 98 °C, an annealing stage lasting 30 seconds at 55 °C, and finally an extend stage lasting 60 seconds at 72 °C. After the 30 cycles, the samples went through a final extension at 72 °C for 5 minutes before cooling down to the holding temperature at 4 °C. Ten ng of the resulting PCR reaction were used from each sample to send for sequencing.

3.0 RESULTS

3.1. Bacteria Isolation

A couple hundred of bacteria colonies grew after plating seawater samples. The colonies that grew varied in size, color, texture, and shape, as well as some appeared to digest the agar, as they would leave impressions within the agar as they grew. Different bacteria were also found to grow at different rates, with some appearing a few days following incubation.

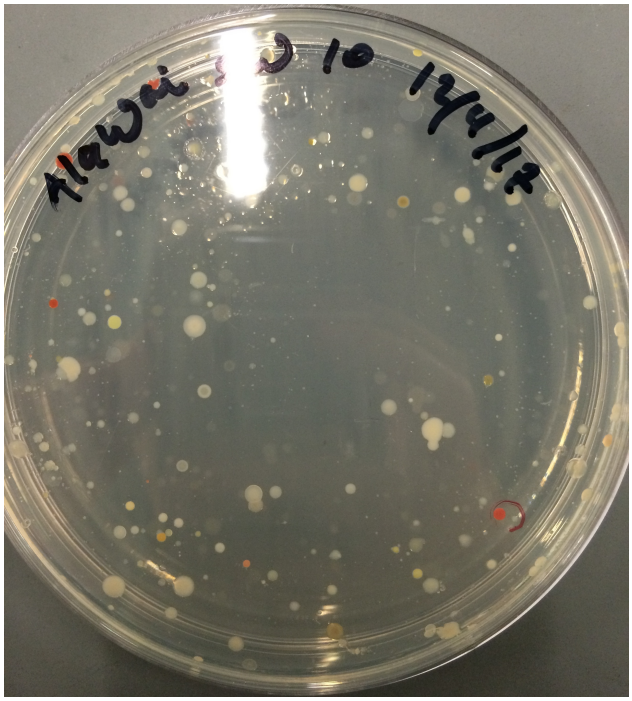


Figure 1: *A photo example of one plate after plating 100 µL of seawater sample.*

3.2. Phage Detection

To find phages by conducting plaque assays, different conditions were used before successfully finding plaque formations on plates. One approach used 200 µL of unfiltered seawater sample combined with 100 µL of dense bacteria culture for plaque assays. Results from these plaque assays contained no plaques. A different approach was

to first filter the seawater sample with a 0.2-micron filter before using 200 µL for plaque assays. Again, these resulted with no plaques. Plaques only appeared after first running the seawater through a 0.2-micron filter followed by inoculation with 200 µL of dense bacteria culture with 10 mL of marine broth and incubated overnight. After overnight incubation, using 100 µL from the inoculated seawater sample for the plaque assay resulted in plaques for a handful of bacteria hosts. Out of 25-isolated bacteria from the Ala Wai canal, 5 isolates were found to be hosts for phages. Bacteria isolates 1, 7, 11, 15, and 18 tested positive with phages.

3.3. Restriction Enzyme Digest and Gel Electrophoresis

Results from the restriction enzyme digest showed that phage isolates from the same bacteria host had the same band pattern on the agarose gel.

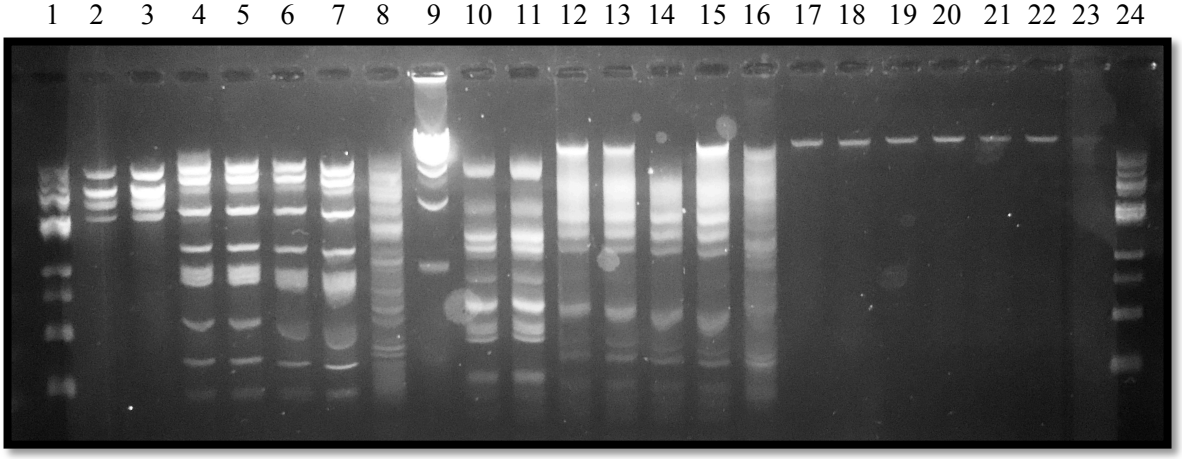


Figure 2: *Image of gel electrophoresis for restriction enzyme digest. Lane 1: 1kb ladder. Lanes 2-3: EcoRI with phage 1-2 and 1-3. Lanes 4-7: EcoRI with phages 7-1,-2,-3,& -4. Lane 8: EcoRI with phage 18-2. Lane 9: 5kb ladder. Lanes 10-11: HindIII with phage 1-2 and 1-3. Lanes 12-15: HindIII with phages 7-1,-2,-3,& -4. Lane 16: HindIII with phage 18-2. Lanes 17-23: controls for respective phage samples. Lane 24: 1kb ladder.*

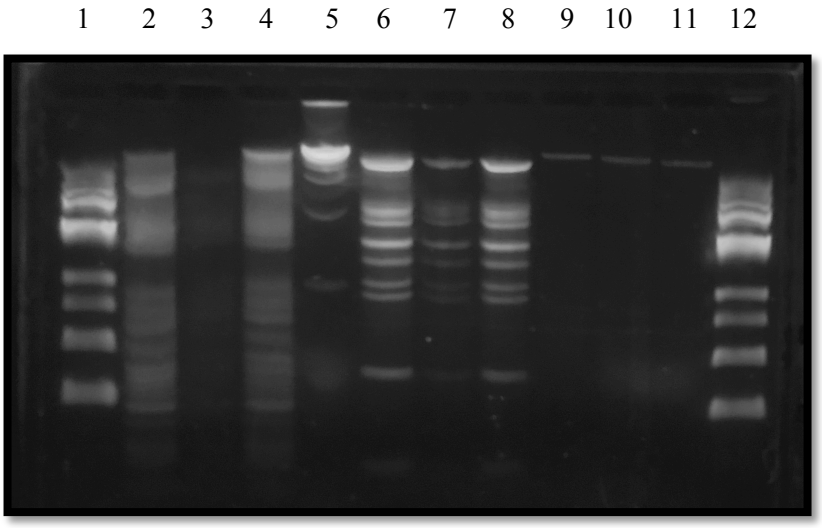


Figure 3: *Image of gel electrophoresis for restriction enzyme digest. Lane 1: 1kb ladder. Lanes 2-4: HindIII with phages 11-2,-3,& -4. Lane 5: 5kb ladder. Lanes: 6-8: EcoRI with phages 11-2,-3,& -4. Lanes 9-11: controls for respective phage samples. Lane 12: 1kb ladder.*

As shown in figures 2 and 3, band patterns for phages from the same host appear to be the same. Additionally, when looking at the band patter for phages to each host, the phages are unique to their respective host.

3.4. Cross-Infection Test

Results from the cross-infection test using the host bacteria isolates with isolated phage samples showed positive for cross-infections using a spot test (phage lysate pipetted directly on to a lawn of host bacteria). As shown in the table below, bacteria 11, 15, and 18 seemed to suggest infection by phages isolated from different hosts, because of what appeared to be turbid plaques. However, the apparent turbid "plaques" could simply be a result of inhibition of bacterial growth by the spotted liquid.

Following up on the cross-infection test, individual plaque assays were done to determine if actual infection was taking place. The results from the plaque assays showed

that the no cross-infections occurred, meaning all isolated phages were specific to their particular host out of the collection of bacteria isolated.

Table 1: *Cross-infection test results based on spot testing. Solid black dots indicate clear plaque formation while clear circles in table indicate turbid plaque formation.*

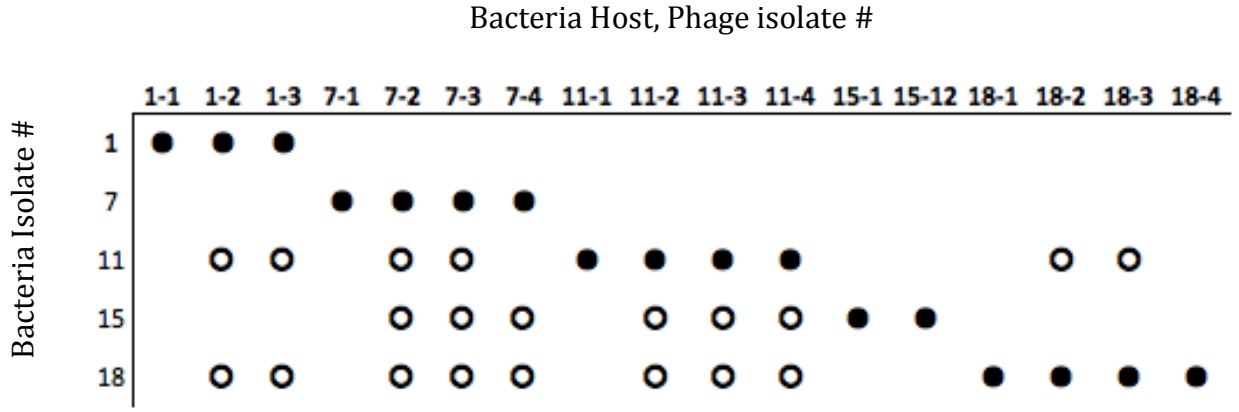
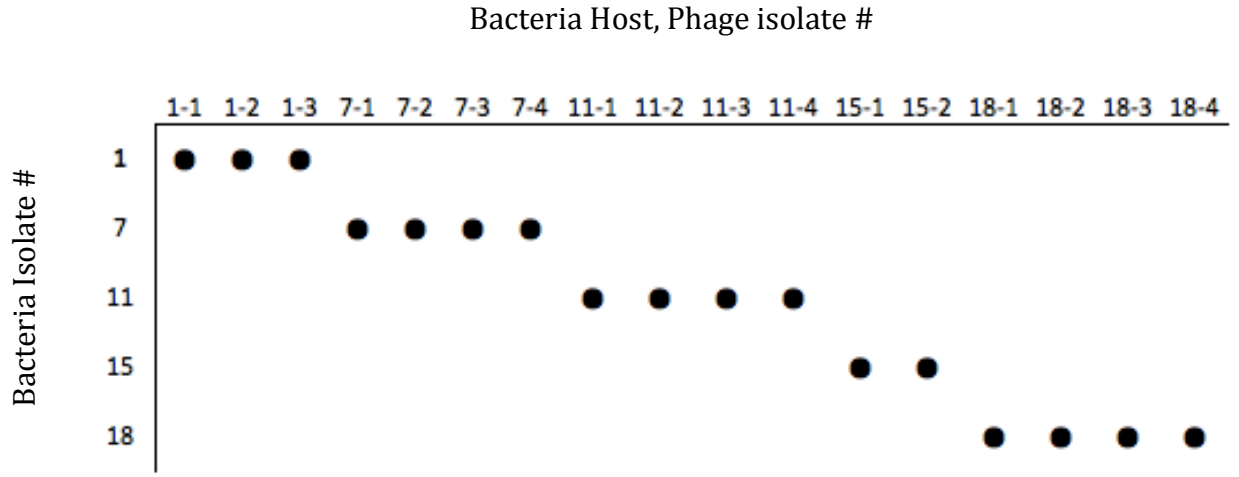


Table 2: *Cross-infection test results after assay. Solid black dots indicate clear plaque formation while clear circles in table indicate turbid plaque formation.*



3.5. Electron Microscopy

Phage-like particles were found through electron microscopy (Figs. 4-8) with one of them (the phage infecting bacterium11) having a tail, suggesting it belongs to the viral family *Caudovirales* (Fig. 6).

Phage from host bacteria 1:

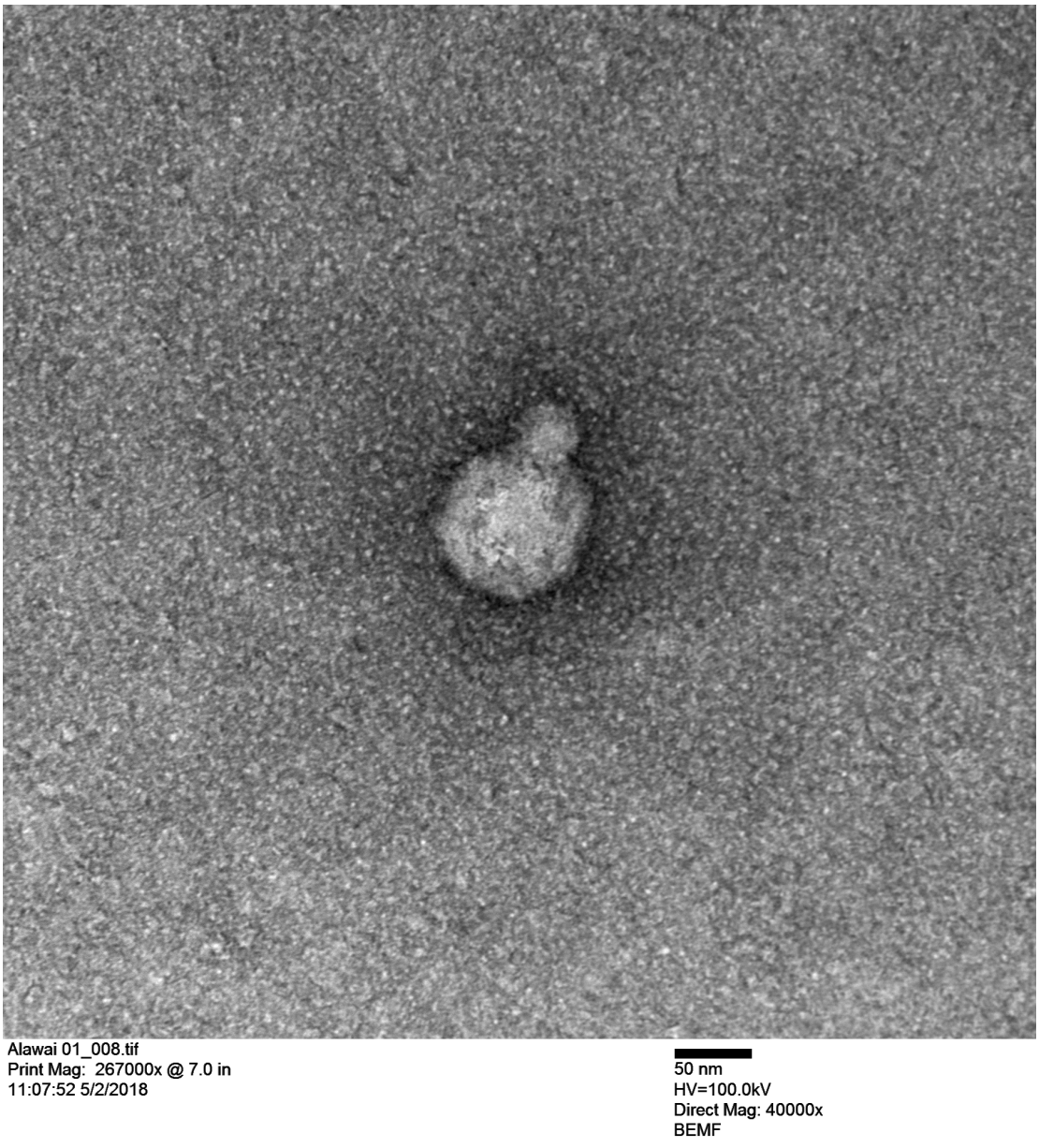


Figure 4: *Phage-like particle from host bacterium 1, viewed at 40,000x magnification. These phages appear to have a bud-like tail.*

Phage from bacteria 7:

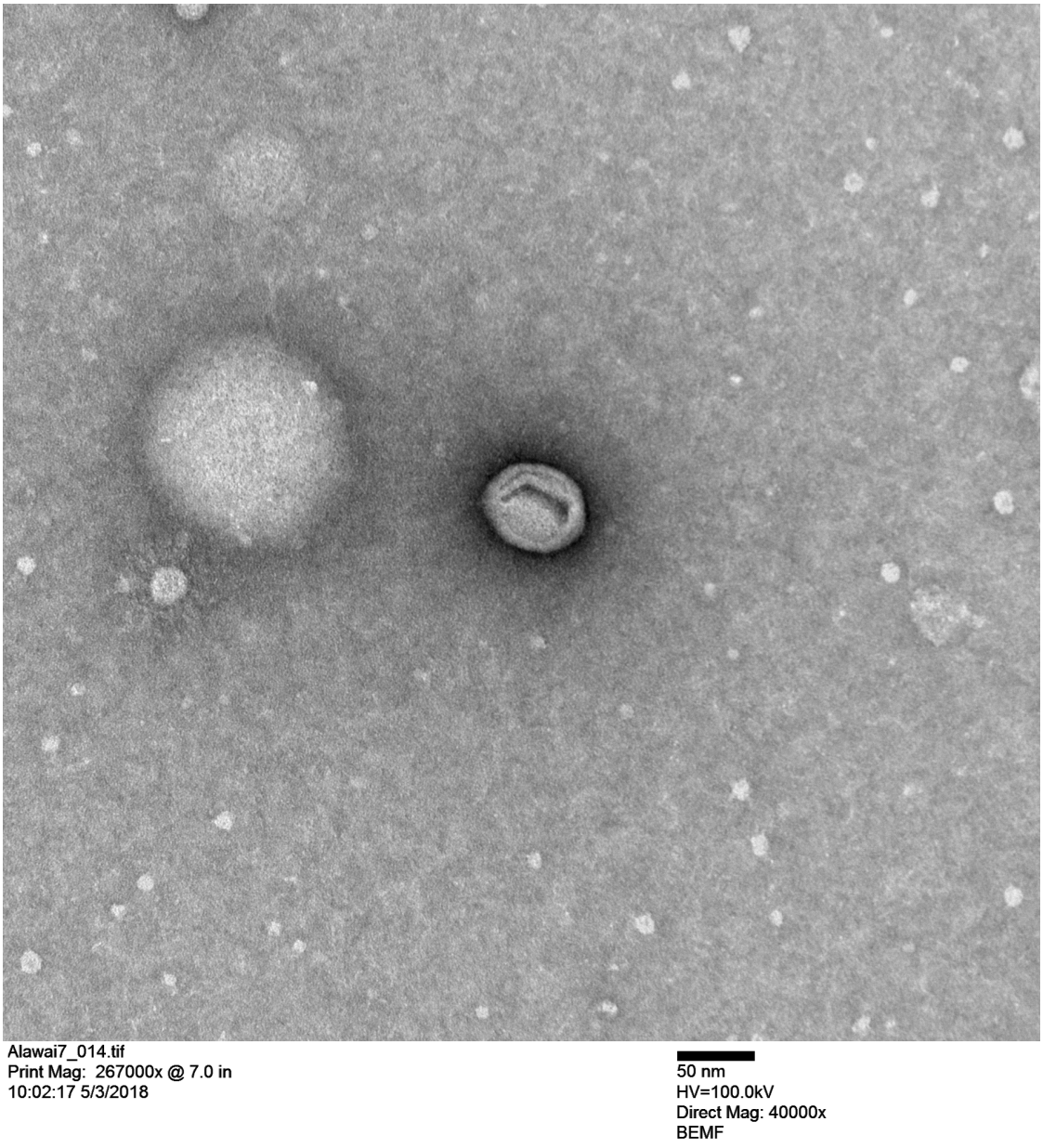


Figure 5: *Phage-like particle from host bacterium 7, viewed at 40,000x magnification.*

Phage from bacteria 11:

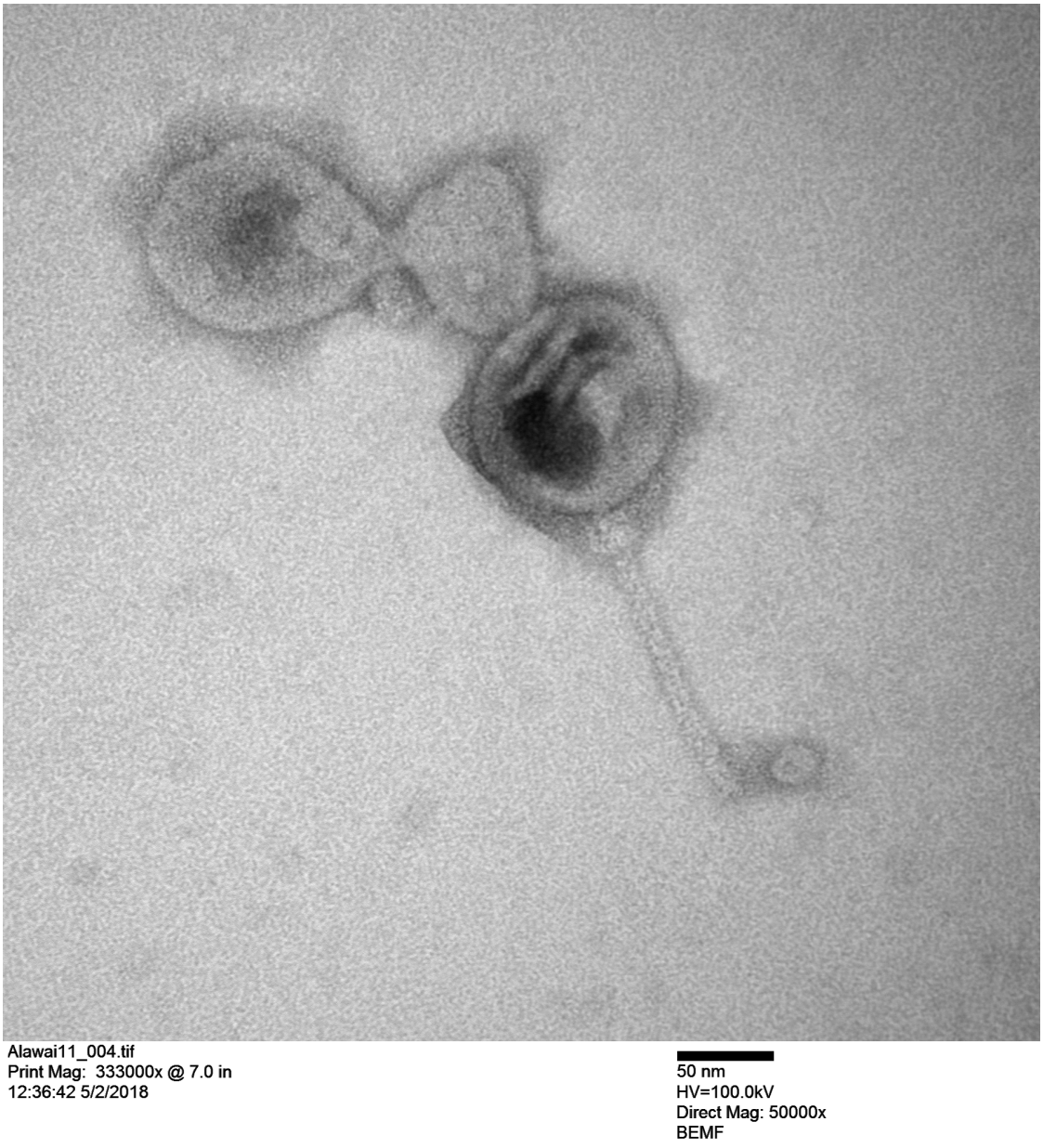


Figure 6: *Phage from host bacterium 11, viewed at 50,000x magnification. These phages have a distinct tail.*

Phages from bacteria 15:

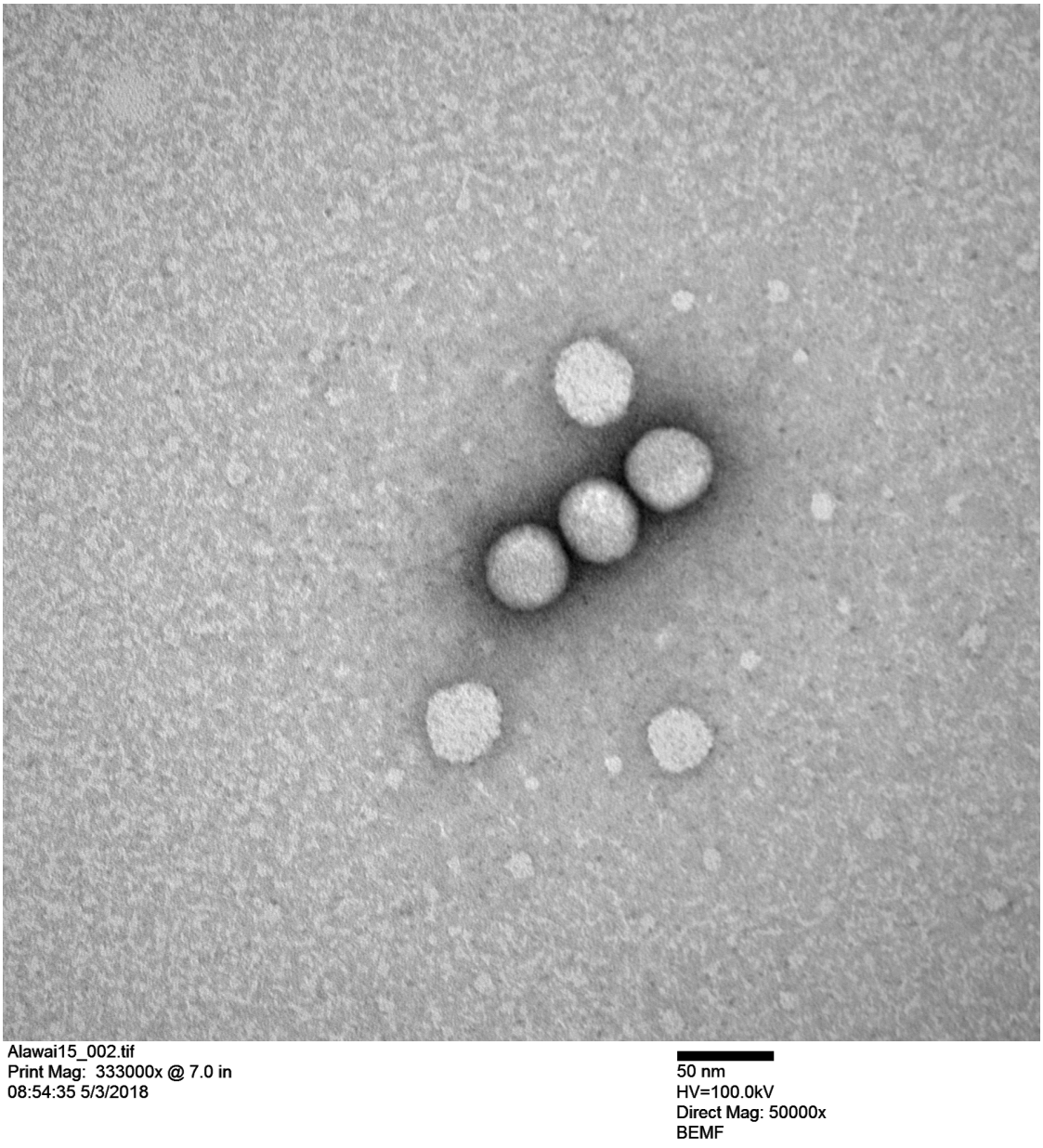


Figure 7: *Phage-like particles from host bacterium 15, viewed at 50,000x magnification. These particles commonly appeared alone or in groups of either two or three, this being an example of a triplet group.*

Phage from bacteria 18:

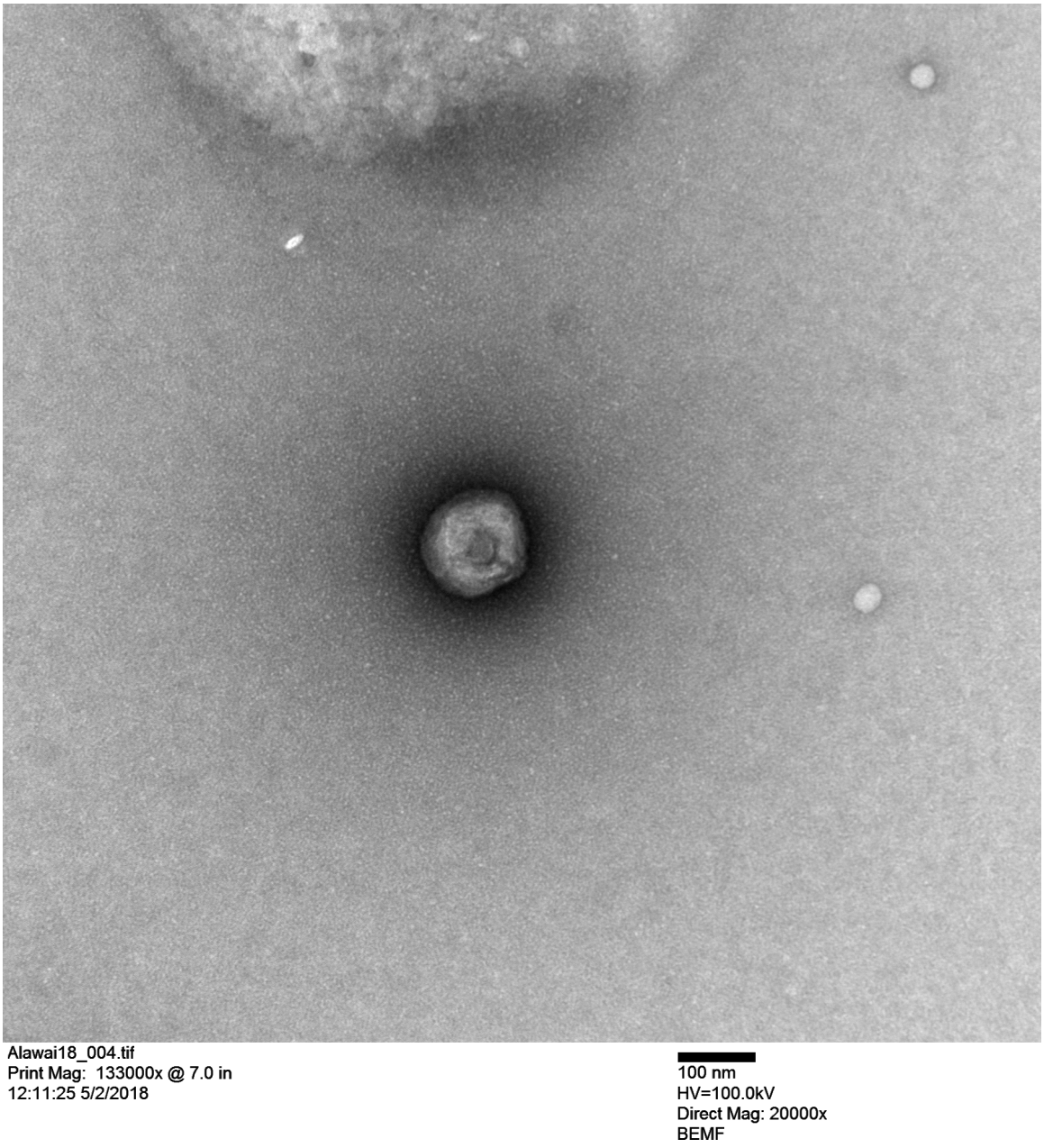


Figure 8: *Phage-like particle from host bacterium 18, viewed at 20,000x magnification.*

3.6. 16S rRNA Gene Sequencing

Results from sequencing showed that the majority of bacteria isolates were in the phylum Proteobacteria (22 out of 26), all of them gammaproteobacteria. Only two belonged to the phylum Firmicutes (both Bacillales) and two the phylum Bacteroidetes (both Flavobacteriaceae). Eight out of the total 25 isolates were in the genus *Vibrio* (9 including the extra isolate), while 7 were in the genus *Pseudoaltermonas*. All other isolates were of a variety different types that can be seen in figure 9. The 5 host bacteria turned out to be of three genera, *Pseudoaltermonas* (bacteria 1 and 18), *Vibrio* (bacteria 7 and 15), and *Serratia* (bacterium 11).

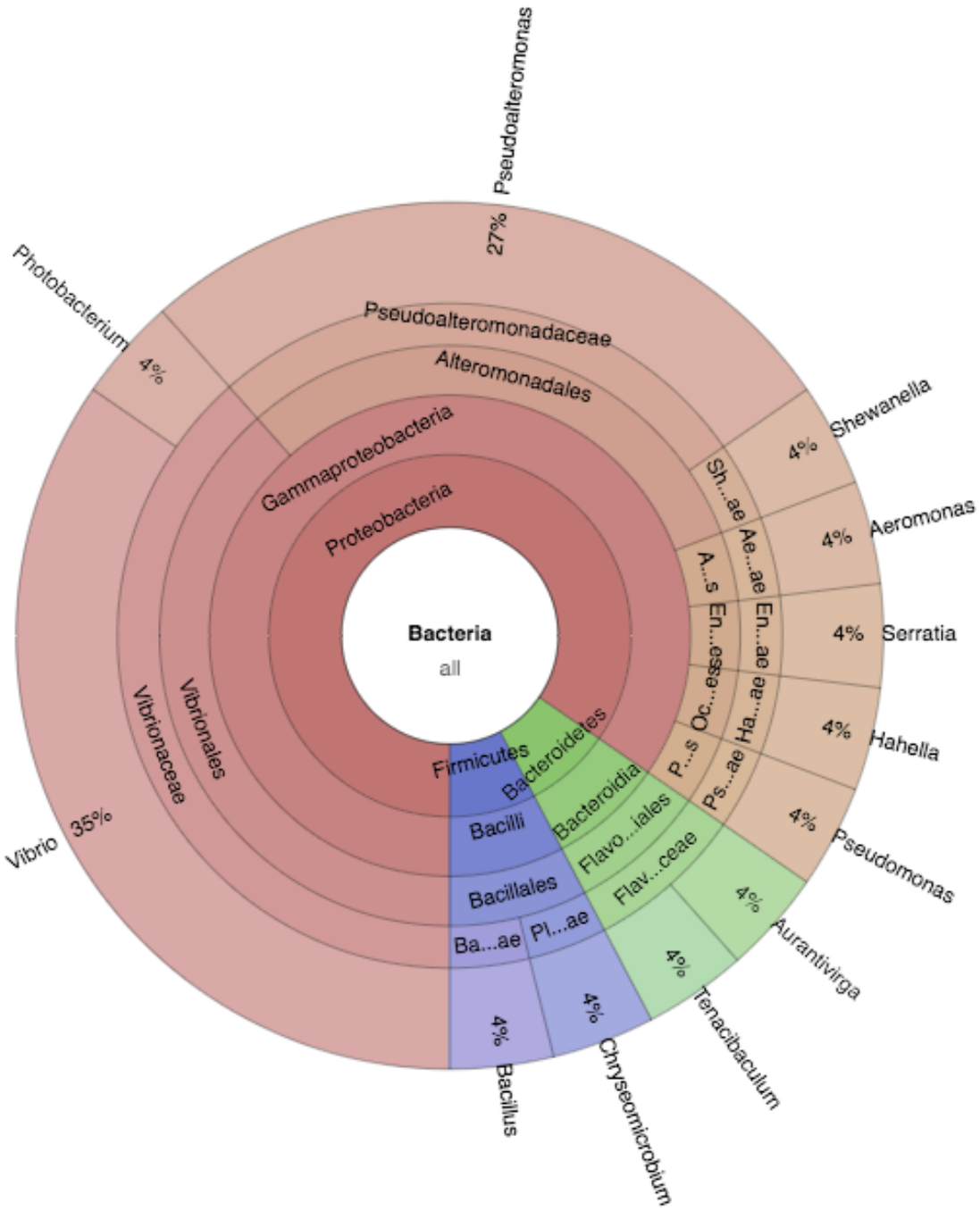


Figure 9: *Hierarchical pie chart of bacteria isolates generated from the SILVAngs website. Percentages are out of a total of 26 isolates (25 from this study and one vibrio isolated previously).*

4.0 DISCUSSION

Our method to find phages involved inoculated filtered seawater with dense bacteria culture, which provided us with information that the phages were initially present in the wild but not information on their abundance. Through this method, as little as one phage was needed to be present due to the fact that the inoculation process stimulates phage replication. Therefore, our data reflects the presence of the phages in the Ala Wai but not their population density per liter of water.

Because we tested for plaques via plaque assays, we were more likely to identify lytic phages than temperate phages. Temperate phages very well could have been present in the water samples collected, however, lytic phages will present much more noticeable plaques compared to temperate phages. Comparatively, another study found also phages that infect *Pseudoaltermonas* after using the same agar overlay technique (Wichels et al., 1998). Future research could also take into account other methods to broaden the phage detection range.

Our data from the restriction enzyme digests suggest that each of the initial different phage picks per bacteria host are actually the same phage. However, the results from the spot tests show that not each of the phage isolates infect the same hosts. This further suggests that there may be small genetic differences that the restriction enzyme digests could not depict that determine the ability to infect certain bacteria.

5.0 CONCLUSION

Though this research only reflects a very small aspect of microbial activity within the Ala Wai, we have been able to establish relationships as well as genetic diversity between phages and bacteria in the canal. The Ala Wai canal is used for many types of recreational activities, including fishing, which puts people at risk to exposure of potentially harmful bacteria. It is possible to use phages that infect bacterial pathogens to treat infected people, known as phage therapy. Future studies could be done to broaden the range of known phage-host interactions within the Ala Wai canal and other water sources around the island to bring more information to the public about the microbes present in the water.

Studies like this could be done around the rest Oahu at various different locations and sites. Identifying areas where common bacteria are located in general could be useful information for people in the area. Additionally, any known marine pathogens could be a potential host for phages and could be used for phage therapy. This could be applied to other organisms, such as coral, that are susceptible to bacteria infection with which phages could decrease stress from bacteria.

6.0 LITERATURE CITED

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